Remarks

A copy of the document WO 98/27220, listed as publication "AA" on the Information Disclosure Statement filed April 19, 2000, is included herewith. We respectfully request consideration of that publication.

The continuing data listed in the first paragraph of the Specification has been updated to include the filing date and the status of each of the applications. Priority to U.S. Patent Application Serial No. 07/852,013 as claimed therein, is included in the Supplemental Priority Data Sheet which was inadvertently omitted from the filing of the originally executed Declaration. We respectfully submit that the intent to claim priority to U.S. Application Serial No. 07/852,013 is clearly manifested in the continuing data section of the Specification.

Claims 1-56 are present in the case.

We note with appreciation the indication of the allowability of Claims 28, 38-50 and 53. We respectfully submit that Claims 50 and 53 should be allowable, since Claim 50 is an independent claim. Moreover, we respectfully submit that Claims 28 and 38-49 should be allowable since Claims 28 and 38-40 have been revised into independent form.

Claims 15, 17, 20-27, 29-34, 51, 52 and 54 have been substantially revised to satisfy 35 U.S.C. §112, second paragraph. Specifically, the term "having" has been deleted from Claim 15. The term "construct" has been replaced with "nucleic acid vector" in Claim 17. Claims 20-25 and 29-34 have been amended to clarify that the claimed vector includes the coding region of the recited gene. The phrase "said the gene" has been omitted from amended Claim 20. Amended Claims 22 and 25-27 recite antecedent basis for each claim element. Claims 51 and 52 as amended recite insertion of the nucleic acid fragment comprising the coding region of the specified gene at the restriction site of the vector. Claim 54 has been amended to depend from Claims 51 and 52, which recite the gene to be

overexpressed.

Turning to the merits of the application, we respectfully submit that Claims 1-6 may be examined over either U.S. Patent No. 5,654,169 ("USP '169") or U.S. Patent No. 5,726,039 ("USP '039"). Claims 1-6 have been amended to clarify that they recite a ribonucleic acid (RNA) molecule as supported by the Specification at page 18, line 1 to page 20, line 12. In sharp contrast, the claims of both USP '169 and USP '039 are drawn to deoxyribonucleic acid (DNA) molecules. Therefore, the invention presented in Claims 1-6 is not the same or even substantially the same as the inventions defined by the claims of USP '169 and USP '039. We thus respectfully request examination of solicited Claims 1-6.

We further respectfully submit that Claims 1-22 and 35-37 are patentably distinct over Goldstein. Specifically, Claims 1-15 have been revised to clarify that they recite RNA molecules. Goldstein makes no mention of even a single RNA molecule. As such, Goldstein fails to anticipate those claims.

Moreover, Claims 16-22 and 35-37 have been sharply amended to distinguish the disclosure of Goldstein. For example, the revision to Claim 16 clarifies that the nucleic acid vector contains a translation-enhancing first nucleic acid fragment derived from a first nucleic acid molecule comprising the first nucleic acid fragment and a first cold shock inducible gene, but that the vector is free from the first cold shock inducible gene. Claim 18 has been amended to depend from Claim 16 and so has been similarly amended. Claim 19 has been amended to recite a vector comprising a cold box, a downstream box, a translation enhancing first nucleic acid fragment and an expression-repressing second nucleic acid fragment wherein when both fragments are derived from the same first nucleic acid molecule, the claimed vectors free from the first cold shock inducible gene. When the first and second nucleic acid fragment of the vector of Claim 19 are derived from different nucleic acid molecules, however, the vectors may contain one of the two cold shock

inducible genes. In other words, Claims 16-22 have been substantially amended to exclude vectors containing naturally-occurring full length cold shock genes. In sharp contrast, Goldstein only discloses a vector containing the naturally-occurring *cspA* gene. Therefore, Goldstein does not anticipate the vectors of Claims 16-22 or the bacteria of Claims 35-37 transformed therewith.

In light of the foregoing, we respectfully submit that Claims 1-56 are in proper condition for allowance, which early action is hereby requested.

Respectfully submitted,

Guy T. Donatiello
Reg. No. 33,167
Attorney for Applicants

GTD:jr
(215) 563-1810

Marked-Up Version Showing Changes Made to the Specification

Page 1, first paragraph:

This application is a continuation of PCT/US99/19030, which is a continuation-in-part of Serial No. 09/293,427, filed April 16, 1999, entitled "Method and Constructs for Inhibiting Protein Expression in Bacteria," which is a continuation-in-part of Serial No. 09/769,945, filed December 19, 1996, entitled "Method and Constructs for Inhibiting Protein Expression in Bacteria," now U.S. Patent No. 5,981,280, which is a continuation-in-part of Serial No. 09/203,806, filed March 1, 1994, entitled "Nucleic Acids Sequence, Stress-Induced Proteins and Uses Thereof," now U.S. Patent No. 5,714,575, which corresponds to parent application Serial No. 07/852,013, filed March 9, 1992, now abandoned. This application also claims the priority of provisional application Serial No. 60/096,938, filed August 20, 1998, entitled "The 5' Untranslated Region of the Cold-Shock *cspA* Gene Regulates Translation Efficiency in Addition to mRNA Stability," and U.S. provisional application Serial No. 60/143,380, entitled "Translational Enhancement by an Element Downstream of the Initiation Codon in *Escherichia coli*."

Marked-Up Version Showing Changes Made to the Claims

1. (Amended) An isolated ~~nucleic~~ribonucleic acid molecule that prolongs the expression of a cold shock inducible ~~genes~~gene under conditions that elicit the cold shock response in a bacterium.

2. (Amended) The ~~nucleic~~isolated ribonucleic acid molecule of Claim 1, wherein said molecule comprises a 5'-UTR of a cold shock inducible gene or a substantially homologous sequence thereof.

3. (Amended) The ~~nucleic~~isolated ribonucleic acid molecule of Claim 2, wherein said 5'-UTR is a 5'-UTR of a cold-shock inducible gene selected from the group consisting of *cspA, cspB and csdA*.

4. (Amended) The isolated ~~nucleic~~ribonucleic acid molecule of Claim 2, wherein said 5'-UTR comprises a cold box or a substantially homologous sequence thereof.

5. (Twice Amended) The ~~nucleic~~ribonucleic acid molecule of Claim 3, wherein said 5'-UTR comprises nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID. NO. 55) or a nucleotide sequence having substantial homology to nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID. NO. 55).

6. (Amended) The ~~nucleic~~isolated ribonucleic acid molecule of Claim 1, wherein said cold shock inducible gene interacts with CspA protein.

7. (Amended) An isolated ~~nucleic~~ribonucleic acid molecule that represses the expression of a cold shock inducible ~~genes~~gene under physiological conditions.

8. (Amended) The isolated ~~nucleic~~ribonucleic acid molecule of Claim 7, comprising at least a portion of the 5'-UTR of a cold shock inducible gene.

9. (Amended) The isolated ~~nucleic~~ribonucleic acid molecule of Claim 8, wherein said cold-shock inducible gene is selected from the group consisting of *cspA, cspB, and csdA*.

11. (Amended) A non-coding ~~nucleic~~ribonucleic acid molecule that enhances the translation of a cold shock inducible ~~genes~~gene under conditions that elicit the cold shock response of a bacterium.

12. (Amended) The ~~nucleic~~ribonucleic acid molecule of Claim 11 comprising at least a portion of the 5'-UTR of a cold shock inducible gene.

13. (Amended) ~~nucleic~~ribonucleic acid molecule of Claim 12 wherein said cold shock inducible gene is selected from the group consisting of *cspA, cspB, and csdA.*

14. (Twice Amended) The ~~nucleic~~ribonucleic acid molecule of Claim 13, comprising nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55) or a nucleotide sequence having substantial homology to nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55).

15. (Amended) The ~~nucleic~~ribonucleic acid molecule of Claim 14 comprising ~~having~~ a sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50.

16. (Amended) A nucleic acid vector that enhances translation of a gene under conditions that elicit a cold-shock response in a bacterium comprising a downstream box and a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment ~~that~~ enhances translation of said first cold shock inducible ~~genes~~gene under conditions that elicit the cold shock response in bacterium, and wherein said nucleic acid vector is free from said first cold shock inducible gene.

17. (Amended) The ~~construct~~nucleic acid vector of Claim 16 further comprising a Shine-Dalgarno sequence.

18. A nucleic acid vector ~~wherein at least a portion of said vector comprises~~of Claim 16 further comprising a cold box, ~~a translational enhancer, and a downstream box~~wherein

said vector ~~directing~~directs prolonged expression and ~~enhancing~~enhances translation of a gene under conditions that elicit a cold shock response in a bacterium.

19. (Amended) A nucleic acid vector that directs the prolonged expression and enhances the translation of a gene under conditions of physiological stress that elicit [the] a cold shock response of a bacterium, and represses the expression of the gene under physiological conditions comprising ~~a cold box, at least a portion of the 5'-UTR of a cold-shock inducible gene that represses the expression of cold-shock inducible genes, at least a portion of the 5'-UTR of a cold-shock inducible gene that enhances translation of cold-shock genes, and a downstream box~~a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under cold shock conditions, a second nucleic acid fragment derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said first or second cold shock inducible gene under physiological conditions, a cold box, and a downstream box, wherein when said first nucleic acid fragment and said second nucleic acid fragment are derived from said first nucleic acid molecule, said vector is free from said first cold shock inducible gene, and wherein when said first and second nucleic acid fragments are respectively derived from said first and second nucleic acid molecules, said vector may comprise one of said first cold shock inducible gene or said second cold shock inducible gene.

20. (Amended) The vector of claim 16 ~~wherein said the gene is a~~further comprising a coding region of a second cold shock inducible gene.

21. (Amended) The vector of Claim 18, ~~wherein said gene is a~~ further comprising

a coding region of a second cold-shock inducible gene.

22. (Amended) The vector of Claim 19, ~~wherein said gene is a~~ further comprising a coding region of a third cold-shock inducible gene, wherein when said first nucleic acid fragment is derived from said first nucleic acid molecule and said second nucleic acid fragment is derived from said second nucleic acid molecule, said third cold shock inducible gene may be one of said first cold shock inducible gene or said second cold shock inducible gene.

23. (Amended) The vector of Claim 16 ~~wherein said gene is~~further comprising a coding region of a heterologous gene.

24. (Amended) The vector of Claim 18 ~~wherein said gene is~~further comprising a coding region of a heterologous gene.

25. (Amended) The vector of Claim 19, ~~wherein said gene is~~further comprising a coding region of a heterologous gene.

26. (Amended) The vector of Claim 16, further comprising a promoter and at least one restriction site downstream of said ~~5' UTR~~first nucleic acid fragment and said downstream box for inserting an additional DNA fragment.

27. (Amended) The vector of Claim 18, further comprising a promoter and at least one restriction site downstream of said ~~5' UTR~~cold box, said first nucleic acid fragment, and said downstream box for inserting an additional DNA fragment.

28. (Amended) ~~The~~A nucleic acid vector ~~of Claim 19 further comprising~~that directs prolonged expression and enhances translation of a gene under conditions of physiological stress that elicit a cold shock response of a bacterium, and represses expression of the gene under physiological conditions comprising a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment enhances translation of said first

cold shock inducible gene under cold shock conditions, a second nucleic acid fragment derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said first or second cold shock inducible gene under physiological conditions, a cold box, a downstream box, a promoter and at least one restriction site downstream of said ~~5' UTR~~cold box, said first nucleic acid fragment, said second nucleic acid fragment, and said downstream box for inserting an additional DNA fragment.

29. (Amended) The vector of claim 26, wherein said additional DNA fragment ~~encodes~~comprises a coding region of a second cold shock inducible gene.

30. (Amended) The vector of Claim 27, wherein said additional DNA fragment ~~encodes~~comprises a coding region of a second cold shock inducible gene.

31. (Amended) The vector of Claim 28, wherein said additional DNA fragment ~~encodes~~comprises a coding region of a cold shock inducible gene.

32. (Amended) The vector of Claim 26, wherein said additional DNA fragment ~~encodes~~comprises a coding region of a heterologous gene.

33. (Amended) The vector of Claim 27, wherein said additional DNA fragment ~~encodes~~comprises a coding region of a heterologous gene.

34. (Amended) The vector of Claim 28, wherein said additional DNA fragment ~~encodes~~comprises a coding region of a heterologous gene.

38. (Amended) A method for overexpressing a gene comprising the steps of: transforming bacteria with a nucleic acid vector ~~of Claim 16~~that enhances translation of a gene under conditions that elicit a cold shock response in a bacterium comprising a downstream box, a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, and a gene,

wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under cold shock conditions, and subjecting said bacteria to conditions that elicit a cold shock response.

39. (Amended) A method for overexpressing a gene comprising the steps of: transforming bacteria with a nucleic acid vector ~~of Claim 18~~ comprising a downstream box, a cold box, a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, and a gene, wherein said vector directs prolonged expression and enhances translation of a gene under conditions that elicit a cold shock response in a bacterium, and subjecting said bacteria to conditions that elicit a cold shock response.

40. (Amended) A method for overexpressing a gene comprising the steps of: transforming bacteria with a nucleic acid vector ~~of Claim 19~~ comprising a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene, a second nucleic acid fragment derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said cold shock inducible gene, a cold box, a downstream box, and a gene, wherein said vector directs prolonged expression and enhances translation of a gene under conditions that elicit a cold shock response of a bacterium and represses expression of the gene under physiological conditions, and subjecting said bacteria to conditions that elicit a cold shock response.

41. (Amended) The method of Claim ~~37~~40, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.

44. (Amended) The method of Claim ~~37~~40, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

50. (Amended) A vector capable of expressing a heterologous gene in a bacterium at physiological temperature or under conditions that elicit a cold shock response comprising regulatory elements in the following order: a promoter, at least a portion of a 5'-UTR of a cold shock ~~protein~~inducible gene, a Shine-Dalgarno sequence, a translational initiation codon, a downstream box ~~of a cold shock inducible gene~~, and at least one restriction enzyme recognition site for insertion of said heterologous gene.

51. (Amended) The vector of Claim 50, further comprising an additional nucleic acid fragment inserted at said restriction enzyme recognition site, wherein said fragment ~~encodes~~comprises a coding region of a cold shock inducible gene and wherein said fragment is regulated by said regulatory elements.

52. (Amended) The vector of Claim 50, further comprising an additional nucleic acid fragment inserted at said restriction enzyme site, wherein said fragment ~~encodes~~comprises a coding region of a heterologous gene and wherein said fragment is regulated by said regulatory elements.

54. (Amended) A method of overexpressing a gene comprising transforming bacteria with a nucleic acid vector of Claim ~~50~~51 or Claim 52 and subjecting said bacteria to conditions that elicit a cold shock response.

Marked-Up Version of the Abstract

Please add the following Abstract. A clean copy of the Abstract is attached on a separate page.

Abstract

Regulatory elements of the 5'UTR of cold-shock inducible genes that prolong the expression of cold-shock inducible genes under conditions that elicit the cold-shock response in a bacterium, repress the expression of the cold-shock inducible genes under physiological conditions, or enhance the translation of cold-shock inducible genes under conditions that elicit a cold-shock response in bacteria; vectors incorporating these elements; and methods for expressing proteins.